PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/47, 31/34, 31/495, 31/425

A2

(11) International Publication Number: **WO 99/64001**

(43) International Publication Date: 16 December 1999 (16.12.99)

(21) International Application Number: PCT/EP99/03827

(22) International Filing Date: 3 June 1999 (03.06.99)

(30) Priority Data:
9812189.0 5 June 1998 (05.06.98) GB

(71) Applicant *(for all designated States except US)*: GLAXO GROUP LIMITED [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BROUWER, Kenneth, Russell [US/US]; Glaxo Wellcome Inc., Five Moore Drive, Research Triangle Park NC 27709 (US). POLLI, Joseph, William [US/US]; Five Moore Drive, Research Triangle Park (US).

(74) Agent: CRAWLEY, Karen; Glaxo Wellcome plc, Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHODS AND COMPOSITIONS FOR INCREASING PENETRATION OF HIV PROTEASE INHIBITORS

(57) **Abstract**

The invention relates to methods for increasing penetration of HIV protease–inhibiting compounds into tissues expressing P–glycoprotein.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

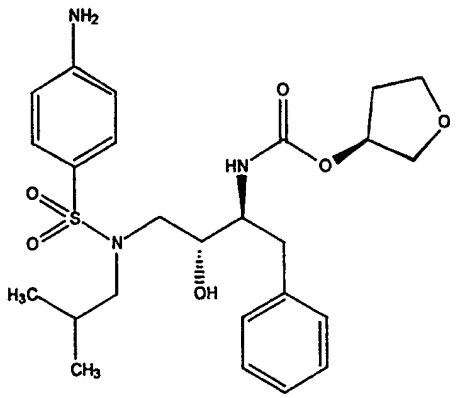
AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

METHODS AND COMPOSITIONS FOR INCREASING PENETRATION OF HIV PROTEASE INHIBITORS

The present invention relates to methods for increasing the penetration of HIV protease inhibitors into the CNS and other P-glycoprotein-expressing tissues such as lymphocytes, testis kidney, liver and placenta. The invention also relates to methods for enhancing the absorption of HIV protease inhibitors from the gastrointestinal tract.

Virus-encoded proteases, which are essential for viral replication, are required for the processing of viral protein precursors. Interference with the processing of protein precursors inhibits the formation of infectious virions. Accordingly, inhibitors of viral proteases may be used to prevent or treat chronic and acute viral infections. Amprenavir (also known as (3S)-tetrahydro-3-furyl N-[(1S,2R)-3-(4-amino-N-isobutylbenzenesulfonamido)-1-benzyl-2-hydroxypropyl]carbamate or [3S-[3R*(1R*,2S*)]]-[3-[[(4-aminophenyl)sulfonyl] (2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-tetrahydro-3-furanyl ester; 4-amino-N-((2syn, 3S)-2-hydroxy-4-phenyl-3-((S)-tetrahydrofuran-3-yloxycarbonylamino)-butyl)-N-isobutyl-benzenesulfonamide; VX-478; 141W94) has HIV aspartyl protease inhibitory activity and is particularly well suited for inhibiting HIV-1 and HIV-2 viruses.

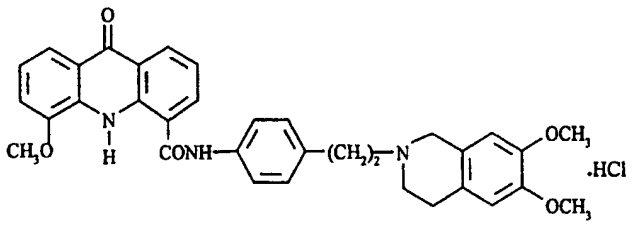
The structure of amprenavir is shown below:



HIV infects the central nervous system (CNS) and may lead to the development of complications, such as AIDS Dementia Complex (ADC). Currently, most HIV protease inhibitors are not effective in the treatment of ADC due to their limited blood-brain barrier (BBB) penetration.

It is known that the poor BBB penetration of pharmaceutical agents is the result of the activity of a multidrug transporter, a membrane-bound P-glycoprotein, which functions as an energy-dependent transport or efflux pump to decrease intracellular accumulation of drug by extruding xenobiotics from the cell. This P-glycoprotein has been identified in normal tissues of secretory epithelium, such as the biliary lining, brush border of the proximal tubule in the kidney and luminal surface of the intestine, and vascular endothelial cells lining the blood brain barrier, placenta and testis. It has been reported that P-glycoprotein limits the oral absorption and brain entry of HIV-1 protease inhibitors (Washington, C.B., et al., Clinical Pharmacology & Therapeutics, 61(2):193, 1997; Kim, R. B. et al. *J. Clin. Invest.* 101(2): 289-294, 1998)

A number of known pharmacological agents have been shown to inhibit P-glycoprotein, including cyclosporin A (also known as cyclosporine), verapamil, tamoxifen, quinidine, d-alpha tocopheryl polyethylene glycol 1000 succinate (Vitamin E-TPGS), VX-710, LY335979, PSC833, and phenothizines. WO 92/12132 and EP 0569380 describe a class of acridine derivatives, in particular 9, 10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-iso-quinolinyl)ethyl]phenyl]-4-acridine-carboxamide (hereinafter referred to as GF120918) or a salt thereof, for use in sensitizing multidrug-resistant cancer cells to chemotherapuetic agents. The structure of GF120918 is shown below:



It has now been discovered that inhibitors of P-glycoprotein can be used to increase the penetration of HIV protease inhibitors, in particular indinavir,

sanquinavir, nelfinavir, ritonavir, and amprenavir into the central nervous system. Inhibitors of P-glycoprotein may also be used to enhance absorption of HIV protease inhibitors from the gastrointenstinal tract and to enhance penetration into other P-glycoprotein expressing tissues such as lymphocytes, testis, kidney, liver, and placenta. Enhanced absorption of HIV protease inhibitors from the gastrointestinal tract, for example, may result in reduced pill burden and/or reduced dose and thereofore reduced toxicity and side effects. In particular, GF120918 or a pharmaceutically acceptable derivative thereof may be used to increase the central nervous system penetration, absorption from the gastrointestinal tract, and penetration into P-glycoprotein expressing tissues of amprenavir.

According to a first aspect of the invention there is provided a method for increasing the penetration of HIV protease inhibitors into the central nervous system by pre-administering and/or simultaneously administering one or more inhibitors of P-glycoprotein. A preferred embodiment of the invention relates to a method for increasing the CNS penetration of amprenavir or a pharmaceutically acceptable derivative thereof by administering one or more inhibitors of P-glycoprotein, in particular GF120918 or a pharmaceutically acceptable derivative thereof.

According to another aspect of the invention there is provided a method for enhancing the absorption of HIV protease inhibitors from the gastrointestinal tract by pre-administering and/or simultaneously administering one or more inhibitors of P-glycoprotein. A preferred embodiment of the invention relates to a method for enhancing the absorption of amprenavir or a pharmaceutically acceptable derivative thereof, from the gastrointestinal tract by administering one or more inhibitors of P-glycoprotein, in particular GF120918 or a pharmaceutically acceptable derivative thereof.

A further embodiment features a method for increasing the penetration of amprenavir or a pharmaceutically acceptable derivative thereof, into P-glycoprotein-expressing tissues, for example, lymphocytes, testis, kidney, liver, and placenta.

A further aspect of the present invention features pharmaceutical compositions comprising one or more HIV protease inhibitors and one or more inhibitors of P-glycoprotein. A preferred embodiment of the invention relates to a pharmaceutical composition comprising amprenavir or a pharmaceutically acceptable derivative thereof and GF120918 or a pharmaceutically acceptable derivative thereof. A further aspect of the present invention features methods of treatment of patients infected with HIV with such pharmaceutical compositions.

GF120918 may be made according to European Patent no. 0569380, or WO92/12132, incorporated herein by reference hereto. A preferred salt of GF120918 is the hydrochloride salt (also known as GF120918A). Pharmaceutical compositions of GF120918 are described in WO96/11007, which is incorporated herein by reference hereto.

Amprenavir may be made according to the methods described in U.S. Patent No. 5,585,397, incorporated herein by reference hereto. A preferred ester of amprenavir is the phosphate ester. Preferred salts of the phosphate ester are the bis-sodium salt and the calcium salt. Preferably, a crystalline form of amprenavir may be used. Pharmaceutical formulations containing amprenavir are described in WO 97/35587, incorporated herein by reference hereto.

The term "pharmaceutically acceptable derivative", as used herein, means any pharmaceutically acceptable salt, ester, salt of an ester, or other derivative of a compound of this invention which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an inhibitorily active metabolite or residue thereof. Particularly favored derivatives and prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) relative to the parent species.

The present invention also provides compositions comprising HIV protease inhibitors and inhibitors of P glycoprotein for use in medical therapy, for example in the treatment of a viral disease in an animal, for example, a human. The

present invention is especially useful for the treatment of diseases caused by retroviruses, such as HIV infections, for example, Acquired Immune Deficiency Syndrome (AIDS) and AIDS-related complex (ARC) as well as diseases caused by hepatitis B and hepatitis C.

The present invention also provides a method for the treatment of a viral infection, particularly an HIV infection, in an animal, for example, a mammal such as a human, which comprises administering to the animal an effective antiviral amount of an HIV protease inhibitor and an inhibitor of P-glycoprotein. Advantageously, the HIV protease-inhibiting compound is amprenavir or a pharmaceutically acceptable derivative thereof and the P-glycoprotein inhibitor, GF120918 or a pharamceutically acceptable derivative thereof.

The combination of the present invention may include other therapeutic agents. Examples of such further therapeutic agents include agents that are effective for the treatment of viral infections or associated conditions such as (1 alpha, 2 beta, 3 alpha)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine [(-)BHCG, SQ-34514], oxetanocin-G (3,4-bis-(hydroxymethyl)-2-oxetanosyl]guanine), acyclic nucleosides (e.g. acyclovir, valaciclovir, famciclovir, ganciclovir, penciclovir), acyclic nucleoside phosphonates (e.g. (S)-1-(3-hydroxy-2-phosphonyl-methoxypropyl)cytosine (HPMPC), adefovir, ribonucleotide reductase inhibitors such as 2-acetylpyridine 5-[(2-chloroanilino)thiocarbonyl) thiocarbonohydrazone, 3'azido-3'-deoxythymidine, other 2',3'-dideoxynucleosides such as 2',3'-dideoxycytidine, 2',3'-dideoxyadenosine, 2',3'-dideoxyinosine, 2',3'-didehydrothymidine, protease inhibitors such as indinavir, ritonavir, nelfinavir, oxathiolane nucleoside analogues such as (-)-cis-1-(2-hydroxymethyl)-1,3-oxathiolane 5-yl)-cytosine (lamivudine) or cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluorocytosine (FTC), 3'-deoxy-3'-fluorothymidine, 5-chloro-2',3'-dideoxy-3'-fluorouridine, (-)-cis-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol (abacavir), ribavirin, 9-[4-hydroxy-2-(hydroxymethyl)but-1-yl]-guanine (H2G), tat inhibitors such as 7-chloro-5-(2-pyrryl)-3H-1,4-benzodiazepin-2-(H)one (Ro5-3335), 7-chloro-1,3-dihydro-5-(1H-pyrrol-2yl)-3H-1,4-benzodiazepin-2-amine (Ro24-7429), interferons such as α-interferon, renal excretion inhibitors such as probenecid, nucleoside transport inhibitors such as dipyridamole; pentoxifylline, N-acetylcysteine (NAC),

Procysteine, α -trichosanthin, phosphonoformic acid, as well as immunomodulators such as interleukin II or thymosin, granulocyte macrophage colony stimulating factors, erythropoetin, soluble CD_4 and genetically engineered derivatives thereof, or non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine (BI-RG-587), loviride (α -APA) and delavuridine (BHAP), and phosphonoformic acid, and 1,4-dihydro-2H-3,1-benzoxazin-2-ones NNRTIs such as (-)-6-chloro-4-cyclopropylethynyl-4-trifluoromethyl-1,4-dihydro-2H-3,1-benzoxazin-2-one (L-743,726 or DMP-266), and quinoxaline NNRTIs such as isopropyl (2S)-7-fluoro-3,4-dihydro-2-ethyl-3-oxo-1(2H)-quinoxalinecarboxylate (HBY1293).

The pharmaceutical compositions of the present invention may be administered by any route appropriate to the condition to be treated, but the preferred route of administration is oral. It will be appreciated however, that the preferred route may vary with, for example, the condition of the recipient.

For each of the above-indicated utilities and indications the amounts required of the active ingredient (as above defined) will depend upon a number of factors including the severity of the condition to be treated and the identity of the recipient and will ultimately be at the discretion of the attendant physician or veterinarian. In general however, for each of these utilities and indications, a suitable effective dose of amprenavir will be in the range of 5 to 100 mg per kilogram body weight of recipient per day, advantageously in the range of 8 to 70 mg per kilogram body weight per day, preferably in the range of 8 to 50 mg per kilogram body weight per day (unless otherwise indicated, all weights of the active ingredient are calculated with respect to the free base of amprenavir).

A suitable dose of GF120918 may be about 0.1 to about 150 mg/kg, preferably from about 5 to about 50 mg/kg of patient body weight, more preferably from about 10 to about 30 mg/kg body weight, most preferably from about 20 to about 30 mg/kg body weight.

The desired dose is preferably presented as one, two, three or four or more subdoses administered at appropriate intervals throughout the day. It is preferable to administer GF120918 with food. These sub-doses may be

administered in unit dosage forms, for example, containing about 25 to 2000 mg, preferably about 25, 50, 150, 200, or 250 mg of active ingredient per unit dose form.

The therapeutic agents of the present invention may be co-administered. "Co-administration" of the P-glycoprotein inhibitor comprehends administration substantially simultaneously with the HIV protease inhibitor (either less than 1 hour before, preferably less than 0.5 hr. before, less than 0.5 hr. after or together), from about 0.5 to about 24 hr. before the administration of the HIV protease inhibitor, or both, i.e. with one or more doses of the same or different P-glycoprotein inhibitors given at least 0.5 hr. before and one dose given substantially simultaneously with (either together with or immediately before or after) the HIV protease inhibitor. Additionally, "co-administration" comprehends administering more than one dose of HIV protease inhibitor within 24 hrs. after a dose of P-glycoprotein inhibitor, in other words, the P-glycoprotein inhibitor need not be administered again before or with every administration or HIV protease inhibitor, but may be administered intermittently during the course of treatment.

While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical composition. The composition comprises the active ingredient as above defined, together with one or more pharmaceutically acceptable excipients therefor and optionally other therapeutic ingredients. The excipient(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

The compositions include those suitable for oral administration and may conveniently be presented in unit dosage form prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing in to association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, sachets of granules or tablets (such as a swallowable, dispersible or chewable tablet) each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored any may be formulated so as to provide slow or controlled release of the active ingredient therein.

The active ingredient may also be presented in a composition comprising micrometer- or nanometer-size particles of active ingredient, which composition may contain other pharmaceutical agents and may optionally be converted to solid form.

Preferred unit dosage compositions are those containing a daily dose or unit daily sub-dose (as herein above recited) or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the composition of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents or taste masking agents.

A further aspect of the invention relates to kits to be used in the treatment of patients suffering from viral infections. These kits include one or more oral

dosage forms of at least one HIV protease inhibitors and one or more oral dosage forms of at least one P-glycoprotein inhibitor or one or more oral dosage forms which comprise both. In a preferred embodiment, the kit comprises amprenavir and GF120918.

By way of illustration, a kit of the invention may include one or more tablets, capsules, caplets, gelcaps or liquid formulations containing the HIV protease inhibitor and one or more tablets,capsules, caplets, gelcaps or liquid formulations containing a P-glycoprotein inhibitor in dosage amounts within the ranges described above. The kits may include as an insert printed dosing information for the co-administration of the agents.

The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way.

Example 1
Preparation of Crystalline Amprenavir

Amprenavir free base was dissolved in a solution consisting of polyethylene glycol 400 (PEG-400), Vitamin E TPGS, and propylene glycol in a ratio of 2:1:0.2, giving a concentration of 19% by weight of amprenavir. To this solution was added a solution of 1:1 water and PEG-400, giving a final concentration of amprenavir of 5-7.5% by weight. Upon standing for 4-24 hours at ambient temperature, fine needles of crystalline amprenavir began to precipitate.

Example 2
Preparation of Crystalline Amprenavir by Seeding

Isopropanol/water: A solution of amprenavir (41.0 kg) in isopropanol (406 L) was concentrated to about 1g/2.5 ml. The solution was cooled to 15-20°C and seeded with crystals of amprenavir from Example 1. The mixture was stirred overnight to allow complete crystallization. Water (151 L) was added slowly while cooling the batch to 5-10°C. The suspension was held at 5-10°C for about one hour. The mixture was then filtered and the solids washed with 76 L of water, followed by 57 L of methyl *tert*-butyl ether. The product was dried in a vacuum oven at 50-60 °C for approximately 18 hours, yielding about 34.8 kg of crystalline amprenavir.

Example 3
Preparation of Crystalline Amprenavir

Amprenavir (from Examples 1 or 2) (0.970 kg) was slurried in 3.88 L, (4.0 vol) of industrial methanol in a 10 L jacketed laboratory reactor. Heating was started with the jacket set at 50 °C. The suspension dissolved when the internal temperature reached 35 °C. The clear solution was heated at 40 °C for 30 minutes to ensure dissolution. Demineralised water (1.94 L, 2 vol) was added over 15 to 20 minutes. The jacket was then set to 20 °C. When the internal temperature reached <25 °C, the solution was seeded with amprenavir (from Examples 1 or 2) (10 g, 1% w/w). The mixture was stirred at 20 °C to 23°C for 1to 10 hours. Water (2.91 L, 3 vol) was then added over 2 hours at ambient

temperature. The suspension was then aged at 10 °C to 12 °C for 1 hour and the resulting crystalline amprenavir was harvested by filtration in vacuo (fast filtration). Crystalline amprenavir was dried in vacuo at 50 °C for 24 hours.

Example 4
Preparation of Amprenavir Acetone Solvate

Approximately 10 g of amprenavir (from Examples 1 or 2) was added to approximately 8 mL of acetone and stirred for several minutes at room temperature. Amprenavir acetone solvate took minutes to hours to precipitate. The mixture, in a closed container, was allowed to stand at room temperature for 2-7 days to allow for crystal ripening. The amprenavir acetone solvate crystals were isolated by vacuum filtering. After air drying for a few hours, the acetone solvate was stored in a sealed glass vial. The acetone solvate was reasonably stable at room temperature for several days to weeks.

Example 5
Preparation of Crystalline Amprenavir from Acetone Solvate

One hundred mg - 200 mg of amprenavir acetone solvate was placed into 2 mL glass vials (*e.g.* Hewlett Packard HPLC vials P/N 5181-3375 with closures P/N 5181-1210) and sealed. The vials were stored at 50 - 60 °C for one month, at which time amprenavir acetone solvate transformed to crystalline amprenavir.

Example 6
Effect of GF120918 on the Transport of HIV Protease-Inhibiting Compounds Across Caco-2 Monolayers

Culturing of Caco-2 epithelial cells. Caco-2 cells were chosen as a model system because this cell line has robust expression of P-glycoprotein (Pgp) and has been used extensively to characterize the transport of new chemical entities. Cells were grown as described by Gan et al. *Pharm. Res.* 10:1722-1725, 1993. For transport studies, Caco-2 cells were seeded onto polycarbonate Transwell™ filter membranes (3.0 μm pore size, 12 mm diameter; Costar, Cambridge, MA) at a density of 60,000 cell/cm^2 and incubated at 37°C, 5% CO_2. The monolayers

were ready for studies 21 days later. Cell culture reagents were purchased from Gibco-BRL, Grand Island, NY. DL-[4-3H]-propranolol (15-30 Ci/mmol) and D-[1-14C]-mannitol (50-63 mCi/mmol) were purchased from Amersham Life Sciences, Arlington Heights, IL.

Transport Studies. Compounds were dissolved at 20 mM in 100% DMSO and dilutions for studies prepared in transport buffer (phosphate buffered saline with glucose, Gibco-BRL #14287-080). In order to determine if Pgp was involved in the transport of these compounds, the HIV protease inhibitors (PIs) were tested at one concentration, two directions (A>B and B>A), and in the presence or absence of the potent, specific Pgp inhibitor, GF120918. The Caco-2 epithelial cell monolayer is "polarized" because of the expression of different apical (gut or "A" side) and basolateral (blood or "B" side) proteins and carriers, such as Pgp. Therefore, one can study the movement of compound from "gut into blood" (A>B) and "blood in to gut" (B>A). Amprenavir, indinavir, ritonavir, and saquinavir were tested at 25 μM, a concentation similar to the peak plasma concentration found in humans after an oral dose.

Transport studies were conducted at 37° C in a humidified incubator over 50 minutes with Transwell™ filter membranes rocked at 110 rpm to minimize the unstirred water layer. Transepithelial electrical resistance (TEER) was measured for each well using an Endohm Meter (World Precision Instruments, Sarasota, FL). Markers for paracellular ([^{14}C]-mannitol) and transcellular ([^{3}H]-propranolol) transport were included in each well as a controls. The HIV PIs were analyzed by HPLC-UV chromatography using a BDS-Hypersil C18 reverse phase column (Keystone, State College, PA), an isocratic gradient, and a mobile phase of 20 mM ammonium acetate (pH 6.8) and acetonitrile.

Data Analysis: The apparent permeability (Papp) was calculated using the equation: $Papp = V/At(Xr/Xo)$ (definitions: Papp, apparent permeability (nm/sec); V, volume of donor chamber (nm^3); A, membrane surface area (nm^2); t, time (sec); Xr, receiver mass; and Xo, original donor mass.). Data presented is the average apparent permeability (Papp) ± standard deviation. All compounds were assayed in triplicate and Papp values reported as nm/sec.

Table 1. Effect of GF120918 on the Transport of HIV Protease Inhibitors Across Caco-2 Cell Monolayers.

Compound (5μM)	Papp A>B	Papp B>A	BA/AB Ratio	Pgp Substrate
Amprenavir	62.7 ± 1.7	177.3 ± 7.8	2.8	Yes
+GF120918	125.6 ± 6.3	131.6 ±1.6	1.0	
Indinavir	4.4 ±1.2	61.4 ± 7.0	14.0	Yes
+GF120918	27.1 ±1.7	36.3 ± 0.9	1.3	
Ritonavir	67.7 ± 7.7	144.7 ± 3.1	2.1	Yes
+GF120918	79.3 ± 8.5	106.2 ± 6.3	1.3	
Saquinavir	2.0 ± 0.2	46.4 ± 2.6	23.3	Yes
+GF120918	8.3 ± 0.4	16.6 ± 1.2	2.0	

HIV PIs were assayed using Caco-2 cells at 25 μM, in two directions (A>B and B<A) and in the presence or absence of GF120918, a potent, specific inhibitor of Pgp (Table 1 and Figure 1). The data is the average from three monolayers ± standard deviation. The BA/AB ratio is used to determine if there is a direction-dependence of compound transport (Table 1). All the protease inhibitors had a 2 to 23-fold higher transport rate for the B>A direction than the rate for the A>B direction. This provides direct evidence that amprenavir and the other HIV PIs are substrates for Pgp. The addition of a P-glycoprotein inhibitor, for example, GF120918, increased membrane permeation of HIV PIs . In vivo, this would result in enhanced intestinal and CNS penetration.

Example 7

Whole Body Autoradiography of the distribution of amprenavir (141W94) with and without GF120918A

Whole body Autoradiography.

To determine the *in vivo* distribution of amprenavir, whole body autoradiography (WBA) studies were performed. Four male CD-1 mice were administered a single oral 250mg(base)/kg dose of GF120918 once a day for four consecutive days, and 4 male CD-1 mice were administered a single oral dose of dosing vehicle (equal in volume to the GF120918 dose volume) once a day for four

consecutive days. Mice were not fasted prior to dosing. Two hours after GF120918 or dosing vehicle administration on the fourth day, a single oral 50mg/kg dose of ^{14}C-amprenavir was administered to each mouse. Mice were not fasted prior to dosing. Mice were euthanized 2 hours after ^{14}C-amprenavir administration by CO_2 asphyxiation, immediately frozen in a bath of hexane and dry ice, and processed for whole-body autoradiography. The two hour time point was selected because amprenavir had significant body distribution and the majority of circulating radioactivity at this time is still parent compound. Animals were prepared for WBA by embedding in a carboxymethylcellulose solution and freezing at -60°C. Using a Leica cryomacrocut microtome, sagittal sections (40 um) were taken from each animal, freeze-dried, and exposed to ^{14}C-sensitive phosphor-imaging plates. The imaging plates were scanned on a Fuji BAS 2000 Bio-Imaging system and the resulting digital images were quantitated using Imaging Research MCID/M2 image analysis software (Imaging Research, Ver. 2.0).

Animals treated with GF120918 (Figure 2, Panel B) had increased brain and CSF levels of amprenavir related material over vehicle treated mice (Figure 2, Panel A). This result provided unequivocal evidence that amprenavir has limited CNS penetration in vivo due to efflux by Pgp and that the penetration may be enhanced by GF120918.

Animals treated with GF120918 had a 7.5-fold increase in brain/blood ratio and a 2.5-fold increase in CSF/blood ratio of amprenavir related material compared to vehicle controls (figure 3).

The application of which this description and claims form part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. They may take the form of product, composition, process or use claims and may include, by way of example and without limitation, one or more of the following claims.

CLAIMS

1. A combination of 9, 10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-4-acridinecarboxamide or a pharmaceutically acceptable derivative thereof and amprenavir or a pharmaceutically acceptable derivative thereof.

2. A combination according to claim 1 wherein the pharmaceutically acceptable derivative is the phosphate ester of amprenavir or a salt thereof.

3. A combination according to claim 2 wherein the salt is the bis-sodium salt or the calcium salt of the phosphate ester of amprenavir.

4. Use of 9, 10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-4-acridinecarboxamide or a pharmaceutically acceptable derivative thereof for the manufacture of a medicament for use in increasing the central nervous system penetration of amprenavir or a pharmaceutically acceptable derivative thereof.

5. Use of 9, 10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-4-acridinecarboxamide or a pharmaceutically acceptable derivative thereof for the manufacture of a medicament for use in increasing the central nervous system penetration of amprenavir or a pharmaceutically acceptable derivative thereof.

6. A method of increasing the central nervous system penetration of an HIV protease inhibitor in an HIV-infected host comprising administering to said HIV-infected host an inhibitor of P-glycoprotein and an antivirally effective amount of an HIV protease inhibitor.

7. A method of increasing the absorption of an HIV protease inhibitor from the gastrointestinal tract in an HIV-infected host comprising administering to said host inhibitor of P-glycoprotein and an antivirally effective amount of an HIV protease inhibitor.

8. A method according to claim 6 or 7 wherein the HIV protease inhibitor is selected from the group consisting of saquinavir, ritonavir, nelfinavir, indinavir, and amprenavir.

9. A method according to claim 6 or 7 wherein the inhibitor of P-glycoprotein is selected from the group consisting of cyclosporin A (also known as cyclosporine), verapamil, tamoxifen, quinidine, d-alpha tocopheryl polyethylene glycol 1000 succinate , VX-710, LY335979, PSC833, and phenothizines, and 9, 10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-4-acridine-carboxamide.

10. A method of increasing the central nervous system penetration of amprenavir or a pharmaceutically acceptable derivative thereof in an HIV-infected host comprising administering to said HIV-infected host an inhibitor of P-glycoprotein and an antivirally effective amount of amprenavir or a pharmaceutically acceptable derivative thereof.

11. A method of enhancing the absorption from the gastrointestinal tract of amprenavir or a pharmaceutically acceptable derivative thereof in an HIV-infected host comprising administering to said HIV-infected host an inhibitor of P-glycoprotein and an antivirally effective amount of amprenavir or a pharmaceutically acceptable derivative thereof.

12. A method according to claim 10 or 11 wherein the inhibitor of P-glycoprotein is selected from the group consisting of cyclosporin, verapamil, tamoxifen, quinidine, phenothiazine, d-alpha tocopheryl polyethylene glycol 1000 succinate, VX-710, LY335979, PSC833, and 9, 10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-4-acridine carboxamide.

13. A method according to claim 10 or 11 wherein the inhibitor of P-glycoprotein is 9, 10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-4-acridinecarboxamide.

14. A pharmaceutical composition comprising amprenavir or a pharmaceuticallly acceptable derivative thereof, 9, 10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl]-4-acridinecarboxamide or a salt thereof, and at least one pharmaceutically acceptable carrier therefor.

15. The composition according to claim 14 for use in medical therapy.

16. Use of the combination according to any of claims 1 to 3 in the preparation of a medicament for the treatment of a retrovirus infection.

17. A pharmaceutical composition according to claim 14 in the form of a tablet or capsule.

18. A pharmaceutical composition according to claim 14 in the form of a suspension.

FIG. 1

Effect of GF120918 on the Transport of HIV Protease Inhibitors Across Caco-2 Cell Monolayers.

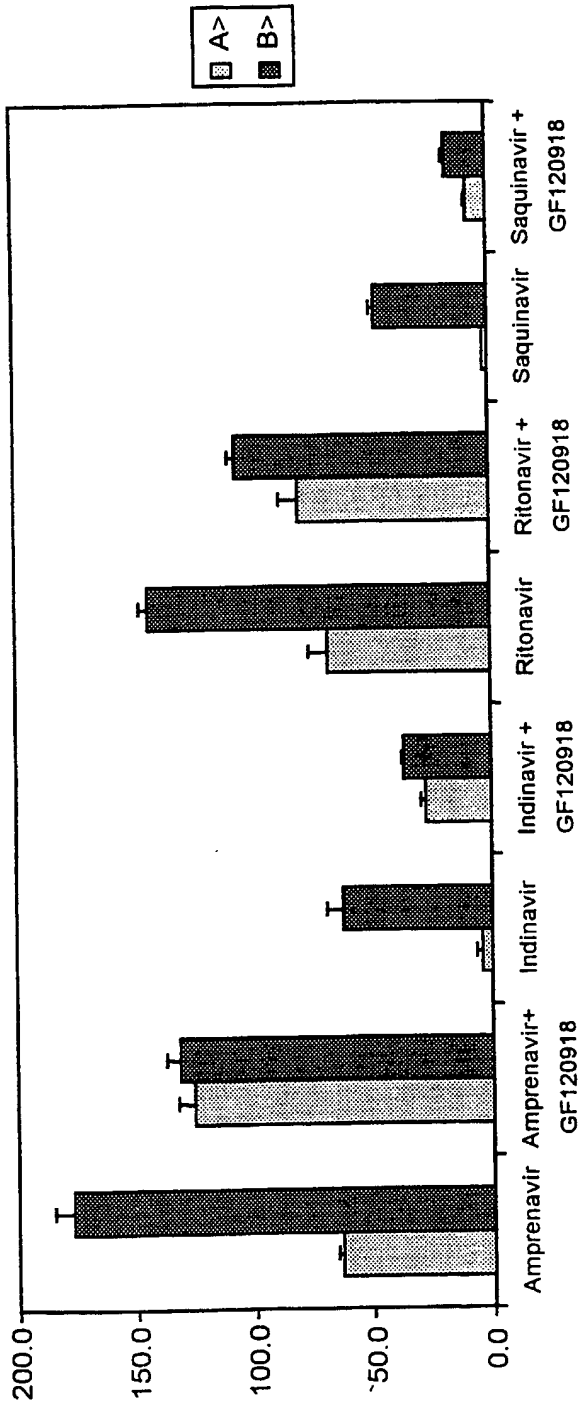


FIG. 2

Distribution of [^{14}C] -amprenavir (141W94) -related material (amprenavir and its metabolites) 2 hours after single oral 50mg (base)/kg dose in male CF-1 with and without co-administration of GF120918.

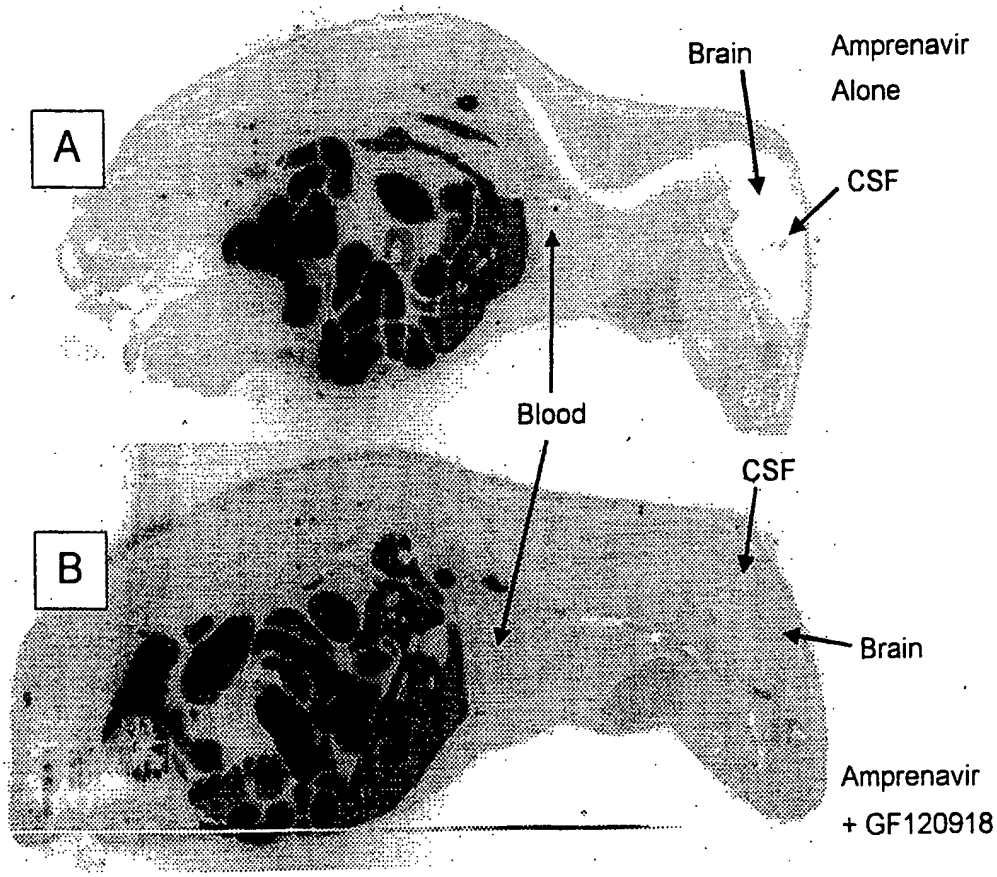
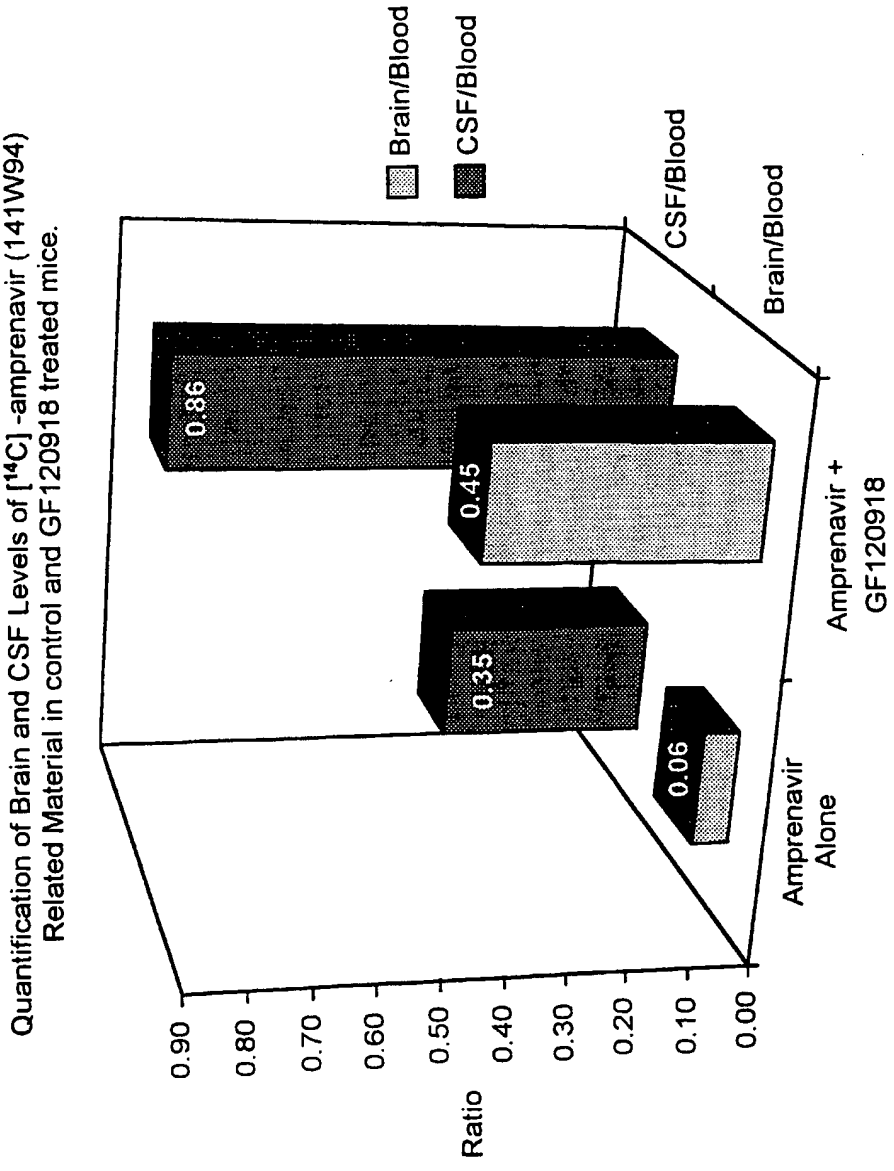


FIG. 3


Quantification of Brain and CSF Levels of [14C] -amprenavir (141W94)
Related Material in control and GF120918 treated mice.
Brain/Blood
CSF/Blood
0.86
0.45
0.35
0.06
Ratio
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Amprenavir Alone
Amprenavir + GF120918